国際セッション口演

（C会場）

5月26日（金） C会場　14：40～15：50

IS-01

Chemokine in inflamed periodontal tissues activates healthy periodontal-ligament stem cell migration

Jong-Bin Lee

Keywords: Chemokines, Homing, Migration, Periodontal stem cells, Tissue regeneration

Aim: The present study aimed to characterize the expression pattern of chemokines obtained from inflamed periodontal defects and to determine the characteristics of human periodontal-ligament stem cells (hPDLSCs) migrated by each specific chemokine.

Materials and Methods: Both inflamed and healthy periodontal tissues were obtained from periodontitis patients (n=11), and the chemokine expression levels were analyzed. The periodontal-tissue-specific chemokines were applied to healthy hPDLSCs from extracted teeth (n=3), with FGF-2 acting as a positive control. Cells were separated by selected chemokines using transwell method into migrated/unmigrated hPDLSCs. The characteristics of the hPDLSC subpopulation recruited by each chemokine were assessed, and gene expression pattern was analyzed by microarray.

Results: Chemokines were categorized into three groups by specific patterns of "appearing," "increasing," and "decreasing/disappearing" from healthy to inflamed tissues. A representative chemokine from each group enhanced the capacities for colony formation and osteogenic/adipogenic differentiation while maintaining the surface markers of hPDLSCs. RANTES/CCL5 significantly increased the cellular migration of hPDLSCs, via enhancement of signaling pathways, regulation of the actin skeleton, and focal adhesion.

Conclusion: The present study found a specific chemokine profile induced by inflammation in periodontal tissues, with RANTES/CCL5 appearing to play a role in the migration of hPDLSCs into inflammatory periodontal lesions.

IS-02

Ultrathin 2D Titanium Carbide MXene (Ti3C2Tx) Nanoflakes Activate WNT/HIF-1-Mediated Metabolism Reprogramming for Periodontal Regeneration

Cui Di

Keywords: hPDLCs, metabolic reprogramming, molecular dynamics simulations, MXenes, periodontal tissue engineering

The regeneration of periodontal defects caused by severe periodontitis has remained a tremendous clinical challenge. The Achiles heel is to promote proliferation and differentiation of periodontal cells. The present study investigates the effect of 2D titanium carbide MXene Film ($Ti_3C_2T_x$) on the osteogenic differentiation of human periodontal ligament progenitor cells (hPDLCs) and regeneration of periodontal defects, and explores metabolic changes as well as the HIF-1α-wnt/β-catenin signaling pathway involved. $Ti_3C_2T_x$ exhibites satisfactory biocompatibility both *in vitro* and *in vivo*. Meawhile, the $Ti_3C_2T_x$ supplement induces the osteogenic differentiation of hPDLCs and upregulates periodontal regeneration-related molecular expression, including alkaline phosphatase (ALP) activity, runt-related transcription factor 2 (RUNX2) and osteopontin (OPN). Surprisingly, distinct metabolomics with metabolic reprogramming and enhanced HIF-1α and β-catenin expression are detected in hPDLCs stimulated by the 2D nanoscale. The potential therapeutic application of $Ti_3C_2T_x$ in periodontal tissue regeneration significantly increases newly-formed bone and inhibited osteoclast formation. In conclusion, this study demonstrates $Ti_3C_2T_x$ could modulate hPDLCs differentiation and periodontal tissue regeneration though HIF-1α-wnt/β-catenin signaling pathway. The underlying mechanism may relate with metabolic reprogramming. (This study was supported by the National Natural Science Foundation Project (No. 81771078, 81970939), Nanjing Clinical Research Center for Oral Diseases (No. 2019060009), and the Jiangsu Provincial Medical Innovation Team (No. CXTDB 2017014))

IS-03

A randomized controlled trial on the effectiveness of the newly invented Salvadora persica Toothbrush and Salvadora Persica Chewing Stick

Nik Madihah Nik Azis

Keywords: miswak, periodontal disease, natural products, chewing stick, toothbrush

The use of Salvadora persica (miswak) chewing sticks as a customary oral hygiene tool has been recommended by the World Health Organization (WHO). The newly invented S. persica toothbrush is designed to merge the ease of use with the beneficial properties of S. persica preserved in its bristle. This randomised control trial aimed to compare the clinical effectiveness between S. persica toothbrush, S. persica chewing stick and the standard toothbrush in plaque and gingivitis control. 78 participants were randomly divided into three different groups to use (i) S. persica toothbrush; or (ii) S. persica chewing stick; or (iii) standard toothbrush in a standardized manner for three consecutive weeks. The Plaque Index and the Periodontal Inflamed Surface Area as well as patient-related outcomes were assessed. There was an improvement of both the oral hygiene level and the severity of gingivitis for all three groups from baseline to three-week post-intervention period. No difference was found between the different types of oral hygiene tools. Following standardised techniques, S. persica oral hygiene tools are as effective as the standard toothbrush in plaque control and gingival health.

IS-04

In vitro study of nano-hydroxyapatite/chitosan gelatine paste as in-situ injectable scaffold

Nadhia Anindhita Harsas

Keywords: nano-hydroxyapatite, chitosan, gelatine, injectable scaffold, bone graft paste

Background: Periodontal and peri-implant tissue defects frequently have limited access, which increases the demand for bone grafts that can be injected and act as scaffolds in the affected area. The limitations of autograft, allograft, and xenograft have increased interest in the utilization of composite materials like hydroxyapatite and other polymers.

Objective: This study aims to evaluate the potential of nano-hydroxyapatite-chitosan gelatine (nHA-KG) bone graft paste as an injectable scaffold.

Method: Injectable scaffolds were made of nano-hydroxyapatite in various concentrations (9, 12, and 15%), chitosan and gelatin (2%). Injectable scaffolds underwent a series of swelling, biodegradability, and pH testing while submerged in a simulated body fluid. The Fourier Transformed Infrared Spectroscopy (FT-IR), and scanning electron microscope (SEM-EDX) were used to characterize these pastes. MTT assay was used to examine the viability of the cells in MC3T3 cells.

Result: nHA 15%-KG2% paste showed the highest swelling percentage, meanwhile nHA12%-KG2% paste was the most biodegraded paste among the three. With an increase in nHA content, the Ca/P ratio also rises. The MTT assay revealed that none of the pastes had any deleterious effects on the cells viability.

Conclusion: A bone graft paste made of nano-hydroxyapatite, chitosan, and gelatin showed promise as an injectable scaffold.

IS-05

Metallothionein-Zinc axis in periodontal immune responses

Mohammad Tariqur Rahman

Keywords: Zinc, Inflammatory cytokine, Metallothionein, Periodontitis, ELISA

Objectives: Inflammation manifests the boosting stock exchange of immune responses against infection. In that process, metallothionein (MT) trades off between inflammation and immune responses. Periodontal bacterial infection and subsequent inflammatory responses cause bone loss in periodontitis (PD). However, the role of MT in PD is largely unknown. Therefore, this presentation is aimed to highlight the link of Zn and the Zn storage protein MT in periodontal diseases.

Materials and methods: The changes in salivary concentrations of pro-inflammatory (IFN-γ, IL-6, and IL-17) and anti-inflammatory (IL-4 and IL-10) cytokines, and salivary MT analyzed using ELISA. Salivary Zn was analyzed using atomic absorption spectrophotometry (AAS)

Results: An increased amount of salivary pro-inflammatory cytokines were observed in the in patients with PD. However, Zn concentration was found lower in the same group of patients compared to the control group ($p<0.05$). Notably, a higher MT/Zn ratios compared to the control group ($p<0.05$) were observed in PD patients.

Conclusions: Both the infection by the periodontal pathogens and bone loss during PD in turn might act as an MT inducer in neighboring soft tissues. Hence, in line with the recent developments in the MT-Zn axis in immune responses against infection opens up new avenues of its role in periodontal health.

IS-06

Oral infection with *Porphyromonas gingivalis* induced epithelial barrier molecules alteration with aging

Sarita Giri

Keywords: Epithelial barrier molecules, *P. gingivalis*, Aging, Trans-epithelial resistance, Permeability

Objectives: Both aging and *Porphyromonas gingivalis* (*P. gingivalis*) might be implicated in modifying the age-related alterations of epithelial barrier molecules. We aim to analyze the effect of effect of aging and oral *P. gingivalis* inoculation on gingival epithelial barrier molecules.

Materials and methods: *In vitro*, young and senescence induced (old) primary human gingival epithelial progenitor (HGEPp) were treated with *P. gingivalis* lipopolysaccharides (LPS). In vivo, C57BL/6J mice aged 10 weeks (young) and 80 weeks (old) were divided into 4 groups: young, old, young + *P. gingivalis* and old + *P. gingivalis* inoculation. *P. gingivalis* was orally inoculated thrice a week for 5 weeks. At 30 days after last inoculation, mice were sacrificed to collect samples. The junctional molecules (claudin-1 & claudin-2, e-cadherin, and connexin) were analyzed for mRNA and protein expression by qRT-PCR, western blotting and immunohistostaining. The level of alveolar bone loss and mRNA expression of inflammatory cytokines was analyzed.

Results: There was significant upregulation of claudin-1 and claudin-2 and significant downregulation of e-cadherin and connexin in LPS treated old cells. Alteration of these molecules resulted in pronounced drop in TER and increase in permeability in LPS treated old cells. The mRNA expression of inflammatory cytokines level was significantly higher in *P. gingivalis* inoculated old mice when compared to other groups.

Conclusions: In conclusion, this study illustrates that the oral administration of *P. gingivalis* has more pronounced effect in the gingival epithelial barrier molecules with aging.

IS-07

Functional Roles of RMND5A in Periodontal Tissue Homeostasis

Rahmad Rifqi Fahreza

Keywords: Periodontal tissue homeostasis, RMND5A, Periodontal ligament, Lithium chloride, WNT/β-catenin

Objectives: Periodontal ligament (PDL) plays a critical role in maintaining periodontal tissue homeostasis. We previously generated PDL cDNA library to explore actively expressed genes in human PDL tissue. So far, we have focused on most of the high-ranked genes including PLAP-1, periostin, cathepsin K. RMND5A is one of the few remaining high-ranked genes without disclosing any functional importance in PDL tissue. Subcellular fractionation analysis implies the nuclear localization of RMND5A in various types of cells and RMND5A may participate in cell mitosis. Thus, in this study, we explored the functions of RMND5A for cytodifferentiation, proliferation, and migration of PDL cells.

Materials and methods: 1. PDL cells were seeded and transfected with RMND5A specific siRNA, 2. PDL cells overexpressing RMND5A were constructed then cultured with an osteo-induce medium and LiCl, an agonist of the canonical Wnt signaling. RT-qPCR and western blotting analysis was performed to detect the mRNA and protein expression levels. Osteogenesis ability was asessed by ALP activity, Alizarin red staining, and RT-qPCR. 3. Cell proliferation ability was quantified by MTT. 4. Cell migration ability was observed by performing a wound-healing experiment.

Results: 1. Inhibition of RMND5A suppressed osteogenesis of PDLF cells 2. LiCl strengthened RMND5A for accelerating PDL cell differentiation 3. RMND5A induces PDLF cell proliferation and reinforced by LiCl 4. RMND5A exogenous expression increased the osteogenic and migration ability of PDL.

Conclusions: RMD5A plays important roles in PDL cell differentiation, proliferation and migration by enhancing WNT/β-catenin signaling pathway